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HYBRIDIZATION BETWEEN *BUFO WOODHOUSII* AND *BUFO PUNCTATUS* FROM THE GRAND CANYON REGION OF ARIZONA

Keith Malmos[1], Robert Reed[1], and Bryan Starrett[2]

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Natural hybridization between toads of the genus *Bufo* is common; most accounts involve representatives from the same species group (Sullivan 1986). Species groups within the genus *Bufo* are hypothesized to be monophyletic groups, based on data that include osteology, lab hybridization studies, advertisement calls, and release calls (summarized in Blair 1972a). Intergroup hybrid adults are expected to be relatively more rare in nature because of the low proportion that develop completely (Blair 1972b). Here we report intergroup hybridization between *Bufo woodhousii* (*americanus* group) and *Bufo punctatus* (*punctatus* group; Blair 1972c). Hybrid *B. punctatus* × *B. woodhousii* previously reported from Colorado near Grand Junction were described as "sterile males with atrophied testes" (McCoy et al. 1967). We present evidence that *B. woodhousii* and *B. punctatus* have hybridized at two new localities in Arizona, Coconino Co., and that atrophied testes are not universal in these hybrids. The localities are approximately 3 km upstream from the Colorado River, near Powell Canyon in the Little Colorado River Gorge, and approximately 8 km downstream of the confluence of the Little Colorado River and Colorado River where Lava Creek empties into the Colorado River. We also analyzed specimens collected by S. W. Aitchison in 1973 from Choal Canyon, Coconino Co., approximately 22.5 km NNE of Kaibito; these specimens include putative hybrid *B. punctatus* × *B. woodhousii.* Hybrids from this series are likely the toads that support the comment by Miller et al. (1982) that hybridization between *B. punctatus* and *B. woodhousii* occurs in Grand Canyon National Park.

Toads were identified and analyzed morphologically using methods similar to those of Ferguson and Lowe (1955) and McCoy et al. (1967). Each toad was dissected to determine sex and condition of testes of putative hybrids. Twelve specimens from the Little Colorado River (LCR) site [3 *B. punctatus* (ASU28935-28937), 8 *B. woodhousii* (ASU28939-28946), and the hybrid (ASU28938)], and 15 of the 17 specimens from Choal Canyon (CC) [8 *B. punctatus* (MNA Z6.529-536), 5 *B. woodhousii* (MNA Z6.522-526), and 2 hybrids (MNA Z6.527-528)] were analyzed. The two toads from Choal Canyon excluded from the analysis were too small to evaluate reliably since ontogenetic changes in cranial crest and parotoid gland morphology occur in some toads (Sullivan 1986). Measurements were taken from preserved male toads that were all the size of reproductively mature individuals. A Helios vernier caliper precise to 0.05 mm was used. Body size and parotoid gland variation among species of toads are diagnostic for many species. For the toads we examined, *B. woodhousii* is larger and has more elongate parotoid glands than *B. punctatus*, which is a smaller toad with small, round parotoid glands. We measured snout-vent length (SVL) and parotoid gland length (PL) and width (PW). A ratio of parotoid gland dimensions (PL/PW) was formed to evaluate gland shape.

All toads from the LCR collection have developed gonads and secondary sexual characteristics. The three *B. punctatus* and four *B. woodhousii* males exhibit darkened vocal sacs, well-developed thumb pads, and testes typical for the species. The other four *B. woodhousii* appear to be spent females containing ovaries

[1]Arizona State University, Department of Zoology, Tempe, AZ 85281-1501.
[2]Phoenix Zoo, 455 North Galvin Parkway, Phoenix, AZ 85008.

with undeveloped eggs. The hybrid male possesses one typical looking testis and one greatly enlarged testis, approximately 10 times normal size.

Morphological analysis supports identification of ASU28398 as a hybrid. Values presented are the mean ± SD. The hybrid was 58.80 mm SVL, larger than *B. punctatus* (42.17 ± 1.48 mm) but similar in size to *B. woodhousii* (59.69 ± 6.03). Shape of the parotoid gland, PL/PW, was intermediate for the hybrid, 1.43, relative to *B. punctatus* (1.017 ± 0.053) and *B. woodhousii* (2.161 ± 0.330; Fig. 1). Both PL/PW and SVL are different between the species with at least 95% confidence because the means ± 2STD do not overlap.

Although no specimens were retained from the Lava Creek site (LC), photographs taken in April 1993 provide clear evidence of hybridization between *B. punctatus* and *B. woodhousii* at this second site in Grand Canyon National Park (Fig. 2). Body size, parotoid gland morphology, and coloration of the adult male hybrid are intermediate. *Bufo woodhousii* is larger, has much more elongate parotoid glands, and lacks the spinose red warts seen in *Bufo punctatus*.

We submit this photographic evidence and morphological analysis of toads as support for the suggestion by Stevens (1983) that *B. punctatus* × *B. woodhousii* hybrids occur in the Grand Canyon region of Arizona. We also suggest that, based on specimens not from the Grand Canyon region, but from specimens collected associated with Glen Canyon, Miller et al. (1982) reported that *B. punctatus* × *B. woodhousii* hybrids occur in the Grand Canyon. Whether *B. punctatus* × *B. woodhousii* hybrids from LCR and LC could reproduce would require histological analysis and additional sampling to determine if hybrids have viable sperm. We are, however, unaware of other reports of enlarged testes in hybrid toads.

Three toads from Choal Canyon, MNA Z6.527-528 and MNA Z6.496, may be hybrids based on intermediate values of PL/PW (1.42 ± 0.04). As in the LCR series, SVLs of hybrids (58.01 ± 3.37) are greater than *B. punctatus* (48.84 ± 6.17), but similar to *B. woodhousii* (56.02 ± 7.87; Fig. 1). The means ± 2STD for SVL and PL/PW overlap for the CC sample;

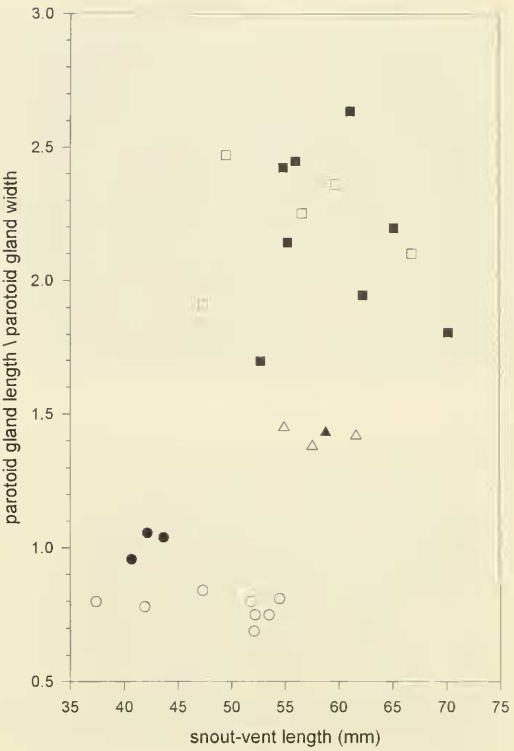

Fig. 1. Comparison of relative sizes and parotoid gland dimensions of specimens from the Little Colorado River locality (closed symbols) and the Choal Canyon locality (open symbols). Circles are *Bufo woodhousii*, squares are *Bufo punctatus*, and triangles are hybrids.

therefore, significant statistical differences do not exist. A small sample size is likely influential. Gonadal development in some CC hybrids is unusual; MNA Z6.496 could not be sexed by its gonads or secondary sexual characters. The other two hybrids, MNA Z6.527 and 528, have darkened thumb pads and vocal sacs. Both testes of MNA Z6.527 appear normal, but MNA Z6.528 has one enlarged testis and the other absent or greatly reduced. Again, whether hybrid males of this cross are reproductively functional is unknown.

Field observations suggest that hybridization at LCR may be relatively common. When the LCR collection was obtained, 13–14 May 1993, advertisement calls typical of *B. woodhousii* and *B. punctatus* were both heard at night, as well as calls that sounded aberrant, approximately intermediate in duration, pulse rate, and pitch of each species. No other species of toads were observed during spring months

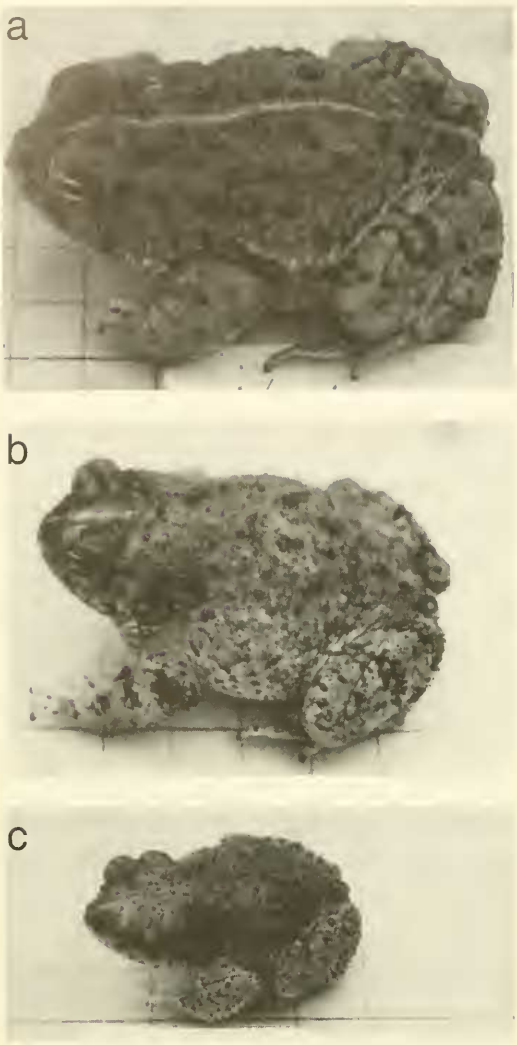

Fig. 2. Photographs of toads from the Lava Creek locality: (a) *Bufo woodhousii*, (b) hybrid, (c) *Bufo punctatus*.

at the LCR site for two years. Advertisement calls produced by hybrid toads often have characteristics intermediate to their parental forms (Blair 1956, Zweifel 1968, Sullivan 1986, 1990). Calls of suspected hybrids were not heard at Lava Creek, but both species chorused together there in April 1993.

Habitat disturbance and environmental change associated with Glen Canyon Dam may contribute to hybridization between these taxa in the Grand Canyon region. Other hybrid zones between toads are associated with river regulation projects or human impacted areas (Sullivan 1986 and examples cited therein). Alterations to the Colorado River have reduced seasonal peak flows, created large daily fluctuations in flow, and dramatically lowered the temperature of the water. Tributaries such as the Little Colorado River and Lava Creek are relatively less affected. Perhaps departure from historic conditions contributes to the likelihood of contact and hybridization between *B. woodhousii* and *B. punctatus* in the Grand Canyon. Other possible explanations for hybridization include natural perturbations that disrupt ecological separation. Also, natural cycles in population size and species range are hypothesized to account for many hybrid zones (Hewitt 1989).

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